A REVIEW OF DIATOM PREPARATION FOR THE LIGHT MICROSCOPE

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Diatoms (Bacillariophyta), unicellular algae with cell walls composed of silica, are very important members of marine and freshwater microalgal assemblages. In many cases, they account for a large proportion of the primary production in freshwater and marine systems (Hutchinson, 1975).

During recent years, diatoms have been used with increasing frequency to assess the condition of inland waters (Lowe, 1972). They are singularly well-suited for this function as they easily can be identified to the species level. The taxonomy of diatoms, based on the ornamentation of the inert silica wall, can be accomplished using a few pertinent taxonomic references such as Weber (1966), Patrick and Reimer (1976, 1966) and Hustedt (1930).

Diatom valves must be properly prepared prior to taxonomic study. Many techniques exist for diatom preparation but there is no one source to which the beginning diatomist may refer. Many techniques are unique to specific labs. This paper will examine most of the methods currently in use.

Although diatoms may be examined as wet mounts, their true morphology can only be seen on frustules cleaned of all organic material and sand.

1. Burn or Incineration Mounts -- Freshwater

Lightly silicified species such as Rhizoselenia and Attheya must be cleaned by this method. Homogenize the sample by sucking the material up and down a pipette, and place 1-2 drops on a coverslip already on a hot plate at low or no heat. Allow the sample to dry slowly; as the material dries, the frustules will become plastered to the coverslip. After the material is dried, increase the heat to incinerate all the soft parts. Although Weber (1966) recommends 1000°F, temperatures in excess of 500-600°F will approach the melting point of silica and must be avoided. It takes 15-30 minutes for a complete incineration. Plankton net samples can be used this way if the samples are relatively clean. Since the organic material burns to blackness, it is best not to use epiphytes this way.

2. Burn or Incineration Mounts -- Marine

Many marine diatom spp such as Skeletonema and Thalassiosira are lightly silicified and incineration of the samples is required. Furthermore, the salt must be removed before the frustules can be studied. If salt is present, a milky-white precipitate will form upon addition of some silver nitrate. There are several methods

to remove the salt. The samples may be taken through a series of alcohol baths or they may be centrifuged, allowing the diatoms to collect at the bottom of the tube and the salt water to be poured off. Centrifugation of any sample is an unsuitable method, however, as it breaks many frustules. A slower but more reliable method is sedimentation with the diatoms allowed to settle out of the sample and the salt water decanted off. Very lightly silicified diatoms may float so the decanted water must be microscopically examined for them. A small amount of material is placed in vials and filled with distilled water. The vials may be tightly capped and rolled between the hands to wash the material. After settling 4-12 hrs, the water is suctioned off. This is repeated 5-10 times. The above method is then followed.

3. Hydrogen Peroxide Method #1

Put the samples into tall beakers and set them in a sink, if possible. Flood with hydrogen peroxide and allow to sit 3 minutes before adding a pinch of potassium dichromate. After 4-5 minutes, there should be a violent colour and temperature change. The solution will boil vigorously, removing all organic materials.

4. Hydrogen Peroxide Method #2

Samples are placed in 100 ml beakers with about 40 ml hydrogen peroxide and some boiling beads. The solution is placed on a hot plate and boiled almost to dryness.

5. Hydrogen Peroxide Method #3

Swift (1967) suggests using 5 drops of 30% hydrogen peroxide per 50 cc sample in quartz test tubes which are then placed 6 cm from a 1200W mercury lamp for 2 hrs.

6. Nitric Acid Method

Acid must always be used under a hood and always with extreme caution. The hot plates should be covered with heavy-duty aluminum foil to protect them. This method is the most efficient and will rid samples of persistent materials like cellulose. Preserved samples must be washed with distilled water before the acid is added, as formalin and nitric acid may explode under these conditions. Batches of samples may be cleaned at once but care should be taken that the beakers are not placed too close together on the hot plates. Splattering may cause cross-contamination of samples. Use 100 ml beakers and about 50 ml acid and boiling beads, bring to the boiling point and let boil almost to dryness. As soon as the beakers are removed from the heat, add a pinch of potassium dichromate to oxidize whatever organic materials are left.

7. Sulfuric Acid Method #1

Water samples may be filtered through Whatman #1 filter paper to collect the diatoms. These frustules are prepared by placing the filter paper in a 300 ml tall beaker and dampening it with

distilled water. Approximately 20 ml sulfuric acid, 50 ml nitric acid and some glass boiling beads are added and the sample boiled on a hot plate until the volume is reduced. After the beaker is removed from the heat, add potassium dichromate as above.

8. Sulfuric Acid Method #2

Hasle and Fryxell (1970) agitate equal volumes of sample and sulfuric acid, then add potassium permanganate until the water becomes purple coloured. Oxalic acid is added dropwise until the sample becomes clear. The authors also suggest centrifugation.

9. Potassium Persulfate Method

Ma and Jeffrey (1978) have developed a method using potassium persulphate (.2-.4 µg/5 ml sample) and a sand bath to clean diatom frustules. When this method is used, the valves of some species remain intact showing the intercalary bands.

After the material is cleaned using methods 3-8, the cooled beakers are filled with distilled water and the diatoms permitted to settle out. This usually takes 4 hrs. The water is decanted and the beakers refilled. This procedure is repeated until the decanted water is clear. This will remove all unvolatized organic material. Care must be used when decanting material for quantitative studies. After the initial few decantations, it is usually best to transfer the frustules to progressively smaller beakers to minimize loss. If there is a skin on top of the water, a drop of it must be examined microscopically before decanting. If many diatoms are seen in this film, the sample must be recleaned.

The decanted material is transferred to labeled shell vials and preserved with a few drops of neutral formalin (1.4ml NaB_4O_7/ liter formalin; $CuSO_4$ to saturate). If long-term storage is required, dip the cork end and part way down the vial into melted paraffin to retard evaporation.

The cleaned diatoms are mounted using Hyrax or some other suitable material. Place a drop of distilled water in the center of a #0 or #1 square coverslip and then one to a few drops of cleaned material. The amount of sample used depends on its concentration. A solution which yields about 10 frustules per field of vision under oil immersion is ideal. Mix the material and the distilled water with a pipette, filling out the edges of the coverslip. Sometimes a wetting agent is added to aid in spreading the material. If the coverslips are soaked in ethanol prior to use, the alcohol will both clean them and aid in diatom dispersal. Alternately, sample and 1 ml distilled water may be mixed in a small beaker. This suspension is placed on coverslips in varying amounts with a dropping pipette to give different sample densities one of which will probably be correct (Heins, 1977). The coverslips should be allowed to dry slowly at low or no heat. If the coverslips are dried too fast, concentric rings of material will form. Examine the coverslips with high-dry magnification before mounting to ascertain that the material is not too dense. With a toothpick,

place a large drop of Hyrax in the center of an ethanol-cleaned 3x1 inch slide. Put the slide on a hot plate. Immediately pick up a coverslip and invert it into the Hyrax. Let the solvent boil off; the coverslip should dance lightly on top of the slide but the Hyrax must not burn (golden-amber colour). Remove the slide from the heat and press the coverslip down evenly and firmly; allow to sit about 30 seconds before testing the Hyrax with a dissecting needle. If the Hyrax is not brittle, reheat the slide to remove the rest of the solvent. When the slides are cooled, remove excess Hyrax with a razor blade, taking care not to lift off the coverslip and use xylene to clean the slide. Two labels should be added: one on the left of the coverslip with the date, sample number, sampling location and any other pertinent information, and another to the right of the coverslip. The taxon of the dominant diatoms or the legend to the ringed and numbered specimens is placed here.

Patrick and Reimer (1966) give detailed instructions for the preparation of slides with only one to a few diatoms. A suitable frustule is transferred to a gelatin-coated coverslip and then mounted in Hyrax as above.

Hart (1957) offers techniques for small quantities of diatoms from invertebrate guts or whale skin while Moller (1967) provides a method of ringing diatoms and recording their positions.

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